Integration of 3D primary tumor drug-profiling with patient-specific drug gene networks for recommending targeted cancer therapies

Simina Boca (ITCR PI - Georgetown University), Timothy Spicer (IMAT PI - Scripps Research Institute)

February 1, 2019
General concepts, parent grant overview, CDGnet tool
What is precision oncology?

Precision oncology (PO) refers to tailoring interventions to patients in ways that go beyond traditional characteristics of age, sex, disease, symptoms etc by considering biomarkers.

Biomarkers may be:

- genetic characteristics: can be either *germline* (inherited, in normal tissue) or *somatic* (in cancer cells but not normal tissue)
- mRNA or protein expression values: refer to expression in tumors, either in comparison to other tumors or to adjacent normal tissues

Often use the term “molecular profiling” (MP) to refer to some test that considers one or more biomarkers.
Tumor molecular profiling

For some tumor types, it is now routine to check for specific molecular features at diagnosis to decide on a targeted treatment plan eg:

- KRAS-wild type (non-mutated) colorectal cancer is treated with EGFR inhibitors (DNA alteration)
- ER+ breast cancer is treated with tamoxifen and fulvestrant, HER2+ breast cancer is treated with trastuzumab (mRNA/protein expression)

In many cases tumor MP is used after a patient has progressed on multiple lines of therapy and/or has few/no therapy options left.

- Patient may then receive an off-label therapy that is prescribed for their alteration in another tumor type
Prioritize targeted therapies using drug-gene networks

We are working on expanding and prioritizing targeted therapy recommendations by creating networks that integrate the following (inputs in orange):

- Specific alterations found in a patient’s tumor
- Patient’s cancer type
- Biological pathways relevant to cancer type, alterations
- FDA-approved targeted cancer therapies and indications
- Drug-gene connections
- Knowledge about activity of alterations/altered gene
Prioritize targeted therapies using drug-gene networks

We are working on prioritizing targeted therapies by creating networks that integrate the following (inputs in orange):

- **Specific alterations found in a patient’s tumor**
  - eg G13V in KRAS, pathogenic PIK3CA mutation
- **Patient’s cancer type**
  - eg Colorectal cancer
- **Biological pathways relevant to cancer type, alterations**
  - eg KEGG
- **FDA-approved targeted cancer therapies and indications**
  - biomarker, cancer type
- **Gene-drug connections**
  - eg DrugBank
- **Knowledge about activity of alterations/altered gene**
  - eg gene is an oncogene
Prioritize targeted therapies using drug-gene networks

How do we include pathway information?

- We specifically look at downstream targets of oncogenes (genes that are constitutively activated in cancer)
- Reasoning behind this: once an oncogene is activated, it only makes sense to target and block genes and proteins that are found downstream of it, as any upstream targeting will be ineffective.
Look downstream of activated oncogenes

Mutated KRAS means patients are not likely to respond to EGFR inhibitors [https://www.cancercommons.org/wordpress/wp-content/uploads/2017/05/kras-mutations.jpg](https://www.cancercommons.org/wordpress/wp-content/uploads/2017/05/kras-mutations.jpg):
Current landing page for CDGnet (may continue being updated)

https://jkanche.shinyapps.io/nfpm
4 ordered categories of therapies for a patient with a given MP from CDGnet

1. FDA-approved drugs for which the input genes/proteins are biomarkers in their tumor type.
2. FDA-approved drugs for which the input genes/proteins are biomarkers in other tumor types.
3. Drugs for which the input genes/proteins are targets or other genes/proteins downstream of the oncogenic input genes/proteins in the pathway corresponding to their tumor type are targets/biomarkers.*
4. Drugs for which the input genes/proteins are targets or other genes/proteins downstream of the oncogenic input genes/proteins in additional cancer pathways (not in their tumor type) are targets/biomarkers.*

* Could be targeted drugs prescribed for their tumor type or other tumor types OR any FDA-approved drug OR any drug in DrugBank.
Supplement overview
Partnered with IMAT team from Scripps Research Institute in Florida who have an R33 grant “Advanced Development and Validation of 3 Dimensional Spheroid Culture of Primary Cancer Cells using Nano3D Technology”

- Primary goal is to use 3D tissue cultures (organoids) in high-throughput screening (HTS) studies of cancer drugs.
- Long-term vision is to obtain tissue from primary tumors, perform screening, then come back with list of drugs/drug combinations.
ITCR-IMAT collaboration

Our collaboration will enable us to integrate CDGnet with HTS data, compare results from CDGnet to results from HTS, come up with drug combinations that should be tested in follow-up experiments.

Specific aims:

1. Integrate the following resources from a technological perspective: 3D tumor HTS data, MP data, biological networks, evidence for targeted therapies.

2. Develop and visualize network models that include 3D phenotypic drug response along with the MP data, tissue-specific pathways, and known evidence for targeted therapies.
ITCR-IMAT collaboration: PDAC tumor with WES

- Filtered to just keep variants with predicted high or moderate impact.
- 20 mutations in 18 genes (data: Tiriac et al, *Cancer Disc.*, 2018)

<table>
<thead>
<tr>
<th>Gene_protein</th>
<th>Data_type</th>
<th>Alteration</th>
<th>Alteration_snpeff</th>
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<tbody>
<tr>
<td>ATR</td>
<td>Mutation</td>
<td>NA</td>
<td>p.Ser1616Ala</td>
</tr>
<tr>
<td>MSH3</td>
<td>Mutation</td>
<td>NA</td>
<td>p.Glu812Gln</td>
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<tr>
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<td>Mutation</td>
<td>p.R263G</td>
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<td>p.Y195H</td>
<td>p.Tyr195His</td>
</tr>
<tr>
<td>PGR</td>
<td>Mutation</td>
<td>NA</td>
<td>p.Ala180Val</td>
</tr>
<tr>
<td>POLE</td>
<td>Mutation</td>
<td>NA</td>
<td>p.Ile2255Phe</td>
</tr>
<tr>
<td>BRCA2</td>
<td>Mutation</td>
<td>NA</td>
<td>p.Gly1500Arg</td>
</tr>
<tr>
<td>ATXN3</td>
<td>Mutation</td>
<td>NA</td>
<td>p.Ala232_Ala234del</td>
</tr>
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<td>Mutation</td>
<td>p.T29M</td>
<td>p.Thr29Met</td>
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<td>Mutation</td>
<td>NA</td>
<td>p.Asp2643Glu</td>
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<tr>
<td>CHD3</td>
<td>Mutation</td>
<td>NA</td>
<td>p.Pro82dup</td>
</tr>
<tr>
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<td>Mutation</td>
<td>NA</td>
<td>p.Cys632Ser</td>
</tr>
<tr>
<td>CHEK2</td>
<td>Mutation</td>
<td>NA</td>
<td>p.Ile200Thr</td>
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</table>
No therapies have any of these alterations as biomarkers in PDAC (Category 1).

There are a number of therapies that are approved for either BRCA2 (olaparib, rucaparib) or PGR biomarker labels, primarily in breast cancer (includes tamoxifen, everolimus, fulvestrant, palbociclib, ribociclib) (Category 2 - see next slide)

- The PGR biomarker is for overexpression, so that may not be translatable for a point mutation.

Of these genes, only KRAS is an oncogene (in PDAC, colorectal, other cancers).

There are number of proteins downstream of KRAS that are biomarkers/targets for FDA-approved targeted therapies for cancers including melanoma, non-small cell lung cancer, thyroid cancer, colorectal cancer, liver cancer.
ITCR-IMAT collaboration: PDAC tumor with WES

Category 2 (approved in other cancer types)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Gene or Protein</th>
<th>Type</th>
<th>Alteration</th>
<th>Tumor in which it is approved</th>
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<td>BRCA2</td>
<td>g.mutation</td>
<td>deleterious</td>
<td>Breast cancer</td>
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<td>LYNPARZA</td>
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<td>deleterious</td>
<td>Ovarian cancer</td>
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<td>BRCA2</td>
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<td>deleterious</td>
<td>Fallopian tube cancer</td>
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<tr>
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<td>BRCA2</td>
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<td>deleterious</td>
<td>Ovarian cancer</td>
</tr>
<tr>
<td>RUBRACA</td>
<td>BRCA2</td>
<td>mutation</td>
<td>deleterious</td>
<td>Primary peritoneal cancer</td>
</tr>
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<td>VERZENIO</td>
<td>PGR</td>
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<td>overexpression</td>
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<td>ARIMIDEX</td>
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<td>overexpression</td>
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<td>AFINITOR</td>
<td>PGR</td>
<td>gene or protein expression</td>
<td>overexpression</td>
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<td>gene or protein expression</td>
<td>overexpression</td>
<td>Breast cancer</td>
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<td>NOLVADEX</td>
<td>PGR</td>
<td>gene or protein expression</td>
<td>overexpression</td>
<td>Breast cancer</td>
</tr>
</tbody>
</table>

(Table uses drug trade names)
ITCR-IMAT collaboration: PDAC tumor with WES

Category 3: downstream of KRAS

11 drugs total

Possible paths:

- KRAS \rightarrow\) BRAF and KRAS \rightarrow\) RAF1 (3 drugs) (sorafenib, regorafenib, dabrafenib)
- KRAS \rightarrow\) BRAF and KRAS \rightarrow\) ARAF \rightarrow\) MAP2K1 (2 drugs) (cobimetinib, trametinib)
- KRAS \rightarrow\) BRAF (1 drugs) (vemurafenib)
- KRAS \rightarrow\) ARAF \rightarrow\) MAP2K1 (1 drug) (bosutinib)
- KRAS \rightarrow\) PIK3CA and KRAS \rightarrow\) PIK3CD (1 drug) (copanlisib)
- KRAS \rightarrow\) RALGDS \rightarrow\) RALA \rightarrow\) RALBP1 \rightarrow\) RAC1 \rightarrow\) NFKB1 \rightarrow\) VEGFA (3 drugs) (bevacizumab, vandetanib, aflibercept)
Current work in progress:

- Will be focusing on category 3, with FDA-approved targeted cancer therapies, along with category 2 recommendations, to come up with drug combinations to test in follow-up experiments.
- Combination therapies should generally attack different pathways/targets.
- Also considering copy number alteration data, which may provide more recommendations.
Questions?

Input from ITCR community is very welcome!

Email: smb310@georgetown.edu
Twitter: @siminaboca